REMARKS

The Invention

Before addressing each rejection in this case, it is important to understand the fundamental advance underlying the claims in this case and the distinctions over the prior art. The office action of October 3, 2003 does not address this advance. A Declaration Under 37 C.F.R. §1.132, by Dr. Garish Sahni, accompanies this Amendment and explains the invention in greater detail.

The inventors have not made a simple "carrier streptokinase (SK)" that includes fibrin binding domains (FBD's) and has plasminogen (PG) activation characteristics indistinguishable from normal SK -- normal SK activates PG immediately without any lag, virtually immediately on contact between the two. Rather, the inventors have made a hybrid protein that becomes active after a lag and in a clot-specific manner as described below in greater detail.

Briefly, the claimed constructs are initially inactive; they are activated by plasmin in the **blood clot** where inhibiting FBD's that inhibit activity are cleaved off. Thus the Applicants' constructs differ from natural SK (natural SK is 'self-activated' and does not depend on plasmin activation) which begins to activate PG **throughout the circulation immediately after injection**. According to the invention, a substance in the target blood clot is required to trigger local activation, thereby avoiding serious systemic effects of immediate activation.

It is also important to recognize that fibronectin domains are structurally and functionally distinct from human plasminogen kringle domains. The Applicants' fusions, unlike those taught by Malke et al., have PG activation characteristics that show an initial time lag of several (5-30) minutes' duration. This **deliberately** engineered property is an important characteristic of the claimed thrombolytic drug, since it allows the agent to home onto its target (a pathological blood clot) without activating the plasminogen systemically throughout the circulation.

We turn now to the individual rejections in the October 3, 2003 office action.

I. Figure Legend

The figure legend for Fig. 7 is amended in this Amendment. The undersigned apologizes for failing to make this amendment in the previous response.

II. Various issues under 35 U.S.C. §112 ¶2

Various objections are raised to the claims. These objections are overcome by the above amendments.

Claim 32 is amended to delete the phrase objected to. A bracket was inadvertently omitted when making this amendment in the previous response. The error is regretted.

III. 35 U.S.C. §112 ¶1

The office action cites the factors set forth in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988). The first factor is "the nature of the invention". On this point, a key finding in the office action is,

> The specification does not reasonably provide enablement for **any** modified hybrid plasminogen activator comprising **any** modified streptokinase and **any** pair of modified fibrin binding domains derived from domains 4 and 5 and 1 and 2 of human fibronectin [emphasis is added]. Page 3, lines 3-6. See also, page 3, liens 9-11 and page 4, lines 8-11.

The rationale for the rejection is even more explicit at page 4 lines 11 et seq. requiring that the specification provide detailed teachings of essentially **every single possible species** within the scope of the claims.

There are two problems with the above key finding that underlies the enablement rejection.

First, the rejection characterizes the invention as covering "any modified hybrid plasminogen activator comprising any modified streptokinase and any pair of modified fibrin binding domains derived from domains 4 and 5 and 1 and 2 of human fibronectin". This statement of the invention ignores the claim language. As amended, the claims specify that the activator exhibits a plasmin-dependent activation mechanism that delays plasminogen activation. The claims also specify that plasminogen activator must include residues 16-383 of human streptokinase. These claim limitations are significant, and a proper analysis under 35 U.S.C. §112 ¶1 must acknowledge these claim limitations.

Second, the law is clear that a claim need not enable all possible species. The Court was clear on this point in *Wands*, where it allowed claims broad enough to cover compositions that had not been made and that were not disclosed in the specification. By requiring the applicants to enable at a detailed level "any" activator species that falls within the claims, the rejection is plainly premised on an error of law. The specification need only enable a reasonable number of examples which demonstrate that the art can practice the invention at the level of claim breadth. In this case, the specification provides three specific examples (an example fused at the N-terminal end, fused at the C-terminal end, or fused at both ends simultaneously). The resultant fusions exhibit the claimed time-delayed, plasmin-activable and localized clot dissolution effect. See the detailed description and figures summarized, for example at page 56 lines 19-21 and in the table at page 57.

As is clear from the accompanying Declaration Under §132, the specification also gives clear guidance as to how to screen for and identify additional species within the claims. The molecular mechanism of the observed lag is steric hindrance by the fused FBD's. So the specification discloses the specific molecular designs that confer the time-lag activation, and there is a scientific rationale for this phenomenon, viz. that the initial time-lag observed is

actually due to a plasmin-dependent mechanism operative in these new forms, in contrast to the well-known observation that activation of the natural SK-PG activator complex is independent of the presence of plasmin (see: Castellino, 1981, Chem. Reviews vol. 81: 431-442).

Teachings in the specification provide those skilled in the art with the information necessary to conduct assays that enable screening for the desired properties and thereby to produce additional species within the claims. The specification teaches that fusion of appropriate FBD's either at the N- or C-terminus (or both, simultaneously) hinders or retards the PG activation process to varying extents. Those fusions should be accomplished through regions that are either inherently flexible (as indeed the C-terminal region of SK is known to be) or, better, through pre-designed, short regions of amino acid residues of appropriate flexibility so as to display suitable propensity for plasmin-mediated scission so that the SK region is "liberated" from the hindering FBDs, albeit after an initial hiatus, to initiate native-like PG activation. There is a mechanism and structural rationale for the invention and the specification provides specific experiments to screen for the desired function.

It simply is inaccurate to say that the specification provides "no guidance as to the parent sequence to be modified" (page 6, lines 6 et seq.). In fact, the claims are quite specific about the parent SK sequences and the FBD sequences to be modified. Moreover, as noted earlier, human SK is well characterized and modification of residues with an expectation of obtaining the desired biological function is clear. The ability to screen for FBD's that cause a lag but are cleaved by fibrin to yield active SK is similarly clear.

In sum, when the *Wands* factors are properly analyzed, enablement is clear. The claims have been narrowed to specify sequences and they are far narrower than the examiner's analysis indicates. *Wands* itself involved screening for desired function, and it would be inconsistent with the analysis in *Wands* to ignore the art's ability to screen for the desired function, once the specification has pointed out that the function can be obtained and it has disclosed methods to

screen and obtain molecules having the function. The nature of applicants' invention as described above is entirely consistent with the current claim breadth. The specification details specific experiments establishing the claimed lag function, as detailed in the Declaration accompanying this Amendment.

The office action also maintains a so-called written description rejection based not on the function of the claimed hybrid molecule (page 9, lines 7-8), but "...because the specification fails to sufficiently describe the structure of the recited hybrid plasminogen activators." (9:11-12). The claims now specify the parent sequences for both components of the hybrid activator. That amendment overcomes the rejection based on lack of structure in the claims.

IV. 35 U.S.C. §103

All claims are rejected under §103(a) as unpatentable over Brown U.S. 5,151,412 in view of Malke et al. U.S. 5,187,098 and further in view of Atkinson et al. U.S. 5,772,996.

The rejection is based on the finding that it would have been obvious to use the recombinant methods of Malke et al. to prepare said fusion protein, which comprises the components of the cross-linked protein of Brown.

The office action cites the following basis for its prima facie case of obviousness.

> Any person of ordinary skill in the art would know that preparation of a recombinant fusion protein comprising the N- or C-terminal fibrin-binding domains of fibronectin fused to streptokinase would be easier and safer to prepare than a molecules comprising said fibrin binding domains cross-linked to streptokinase. Therefore, any person of ordinary skill in the art would be motivated to use the recombinant methods of Malke et al. to prepare said fusion protein, which comprises the components of the cross-linked protein of Brown et al.

In other words, the motivation underlying the rejection is simply that the fusion "would be easier and safer to prepare". The problem with this analysis is that it fails to acknowledge the purpose for which Brown produced a conjugate. Brown's purpose is to produce a carrier for a pharmaceutically active substance to be administered to a patient systemically, yet targeted to particular sites of the body where the conjugate binds with a high degree of selectivity. In the case of SK, Brown seeks a carrier with "a high degree of selectivity for binding to fibrin" (4:24-25). With this purpose in mind, Brown tolerates (perhaps desires) an extremely variable cross-linked product. The Brown method results in cross-linked polypeptides with chemically undefined make-up since the hetero-bi-functional cross-linking can potentially take place between any of the several amino acid residue side-chains in the two proteins. Further, none of the introduced cross-links were defined with respect to their location in either of the participating polypeptide segment.

There is no particular motivation to give up this cross-linked structure. Indeed, since selectivity for fibrin is the goal, one skilled in the art could assume that Brown actually desires the resulting cross-linked structure. In any event, the proposed combination has no motivation in the art. It is not automatically true that recombinant fusions are better for all purposes, and specifically there is no reason to use them for Brown's purposes. Simplicity of manufacture can be a motivation only wheret the resulting product is the same or at least functionally indistinguishable. That is not the case here.

Moreover, the claimed peptide bonds are amenable to cleavage by plasmin, a result that is desirable for the Applicants' purposes (to activate SK), but that was not Brown's goal. There is no reason at all to think that one skilled in the art seeking to accomplish Brown's goal of selective tissue targeting would want to create a linkage that could be enzymatically degraded. Here again, the proposed alteration of Brown has no basis in the art.

Finally, even if there is a prima facie case of obviousness, such a rejection is overcome by unexpected results. The office action has clear legal error in its analysis of unexpected results. Specifically, the action relies on alleged "inherent properties" to support the proposed combination of references and to avoid analyzing the unexpected results. At page 11, line 2 et seq. the office action gives the following rationale for the obviousness rejection,

> The functional features of a protein are inherent to its structure. The delay in activation of the hybrid plasminogen activator made obvious by the teachings of Brown et al. in view of Malke et al, is inherent to said hybrid plasminogen activator.

The above quotation exposes a serious flaw in the rejection – viz, the examiner has ignored entirely the documented and unchallenged unexpected advantages of the invention, detailed above and in the accompanying declaration. Rather than deal with those unexpected advantages, the examiner simply bootstraps the rejection with "inherent" properties of the proposed combination. If this analysis were correct, there would be no place in the law at all for unexpected advantages. This analysis is legal error. See *In re Newell*, 891 F.2d 899, 13 USPQ2d 1248 (Fed. Cir. 1989) where the Federal Circuit reversed the examiner's finding of obviousness based on inherent properties.

> "[A] retrospective view of inherency is not a substitute for some teaching or suggestion which supports the selection and use of ht he various elements in the particular claimed combination.

See also *Jones v. Hardy*, 727 F.2d 1524, 220 USPQ 1021 (Fed. Cir. 1984); *In re Shetty*, 566 F.2d 81, 86 (C.C.P.A. 1977) "Inherency is quite immaterial if...one of ordinary skill in the art would not appreciate or recognize the inherent result."

The reliance on inherent properties of Brown's cross-linked molecules is also factual error. The Examiner has cited Brown et al (Table 6) as supporting the conclusion that the average activity of their conjugate is 38-50% and the examiner therefore concludes that Brown's data establishes low activity at early time-points. However, the data supports the opposite conclusion. The Table 6 (or for that matter, any data in Brown) only shows the specific activity of the conjugate as a function of different units/ml, i.e., it only establishes the dose-dependence of activity. There is

no showing of activity over time in a way that would suggest a lag, delay or altered kinetics of PG activation. Indeed, under Table 7 they show a closely similar compromise in the activity of urokinase upon conjugation, indicating that the cross-linking reaction, in this case also, leads to a similar compromise in the bioactivity of urokinase as well.

It would be highly inappropriate, therefore, to conclude from such data that merely making a conjugate (that too heterogeneous via chemical linkages) *automatically* allows the generation of the coveted property of time-delayed, plasmin-dependent activation; what the data merely establishes is that the intrinsic activity is compromised in average extent, without having any bearing on the alteration of its kinetics or time-course of manifestation. It is relevant also to state here very unequivocally that the type of covalent bonds engendered by Brown et al's chemical procedure are not amenable to cleavage by plasmin, a natural enzyme that recognizes alpha-amino peptide bonds only.

If the initially delayed kinetics of the claimed invention were indeed an intrinsic or inherent property of the fusion/conjugation, a change in the specific activity and/or rate of PG activation would have been expected to occur. Indeed, as detailed in the Declaration, the inventors have experimentally observed, like Malke et al, that the fusion of kringle domains at the N-terminus does not confer such a delayed effect, in direct contrast to the fusion of finger FBD's.

Apparently Malke is not cited for the components of the claimed fusion protein, but instead for its methodology (recombinant fusion). Malke et al. never even perceived the concept of deactivating and subsequently, activating the recombinant SK. Further, they make absolutely no reference to the fact that the deactivation and then reactivation could offer such a significant benefit in this important field of medical sciences, leave alone actually demonstrating this property.

In short, the cited art does not provide any indication of the Applicants' surprising result. That result is detailed in the accompanying Declaration and summarized below.

It is well established that after injection into the blood, natural SK makes a tight 1:1 stoichiometric complex with the plasminogen present in the blood stream. It is this two-molecule complex that is the active proteolytic, plasminogen-activating principle or enzyme, that, in fact is the effective therapeutic molecule (reviewed in Castellino, FJ ,1981, Chemical reviews vol. 81:431). This SK-PG complex rapidly self-activates to SK-plasmin (in which the zymogen, plasminogen has been converted to its proteolytically active form -- plasmin, by an intramolecularly initiated process whose mechanistic details are not yet fully understood); the SK-plasmin complex then further acts (as an enzyme) on un-complexed, circulating substrate PG molecules to convert these into freely circulating plasmin, which dissolves the pathological blood clots in the vascular system as they travel throughout the bloodstream. However, the chief problem with this scenario is that the plasmin destroys several other vitally required blood factors as well, briefly alluded to above, often leading to severe side effects, including uncontrolled internal bleeding.

Hence, it was important to design SK derivatives with minimal side effects and high target specificity i.e. localized action in the vicinity of the blood clot and *not throughout the system.* For this purpose, applicants have designed, produced and characterized new forms of SK that do not get activated immediately but only do so after a distinct time-delay. This property has neither been anticipated nor been shown by the cited art.

The initial time-lag observed in the invention is actually due to a plasmin-dependent mechanism operative in these new forms (as opposed to the well-known observation that activation of the natural SK-PG activator complex is *independent* of the presence of Plasmin i.e. the zymogen, PG, in the activator complex is activated through an autocatalytic process (see: Castellino, 1981, Chem. Reviews vol. 81: 431-442).

The plasmin-dependence of PG activation by the hybrids disclosed in the application is established in Example 8, paragraph 2, showing that, as a result of deliberate depletion of residual plasmin in the PG preparation used in the experiment by pre-adsorption to a specific, plasmin-absorbing affinity material (soybean trypsin inhibitor-agarose), the *in vitro* lag increased significantly, and showing that the removal/diminution of plasmin in the PG preparation to be activated resulted in abolishment/decrease in lag, and conversely, it significantly decreased upon increasing the plasmin concentration in substrate PG. Further, the activation of PG by the hybrids, and concomitant abolishment of the lag, was found to coincide with the cleavage of the fused fibrin binding domains from the SK portion of the hybrid (Example 8). Unlike native SK which forms an activator enzyme with the zymogen (PG) almost instantaneously, the new forms require the presence of plasmin to activate substrate. And since free plasmin is absent in the circulation normally, but present in the blood clots, the activators with such a property, together with strong fibrin affinity, are indisputably improved in terms of their therapeutic properties related to improved localized activation. The presence of these two properties simultaneously in our constructs, and the unique mechanism associated with these, is clearly distinct both from Malke et al's and Brown et al's constructs.

Since the pathological "target" clot is relatively plasmin-rich, it would allow the *in situ* activation of the modified SK molecules ones they home into the clot by virtue of their fibrin affinity. *More importantly, since free plasmin is rare or absent in circulation, the constructs would remain in an inactive state while sojourning the circulating system until their absorption to the clot.*

None of the cited art makes even a vague reference to the role of plasmin present in the vicinity of the fibrin to play a critical role in the activation of the genetically modified recombinant SK. Further, the examiner must appreciate that plasmin can act upon the biological molecule of the instant application developed by recombination technique. However, it just cannot work on a SK-fibronectin conjugate developed by chemical binding.

The altered mechanism of PG activation displayed by the hybrids in our application has powerful implications in their therapeutic applications since free plasmin is rarely present in the circulation as it is rapidly cleared by circulating plasma proteins called Serpents, such as alpha-2 macro globulin and/or alpha-2 antipasti. References in this context may be made to: Gonias, Einarsson and Pizzo, 1982, J. Clin. Invest. vol. 70, 412-423; Rajagopalan, Gonias and Pizzo, 1987, J. Biol. Chem. vol. 262, 3660-3669). Since free plasmin is relatively abundant in the pathological clot due to local activation of the PG bound to the thrombus by the small quantities of intrinsic TPA (Colman, Hirsh, Marder and Salzman [eds]., 1994, Haemostasis and thrombosis, Basic Principles and Clinical Practice., J. B. Lipincott Co., Philadelphia) it allows the *in situ* activation of the modified SK molecules once they home onto the clot by virtue of their strong fibrin affinity. In other words, since free plasmin is rare or absent in circulation, the constructs would remain in an inactive state while sojourning the circulatory system until their absorption to the clot.

Since it takes a few minutes for a molecule to circulate in the body *via* the main blood vessels, the ability to remain transiently inactive (unlike natural SK) until the protein reaches the pathological blood clot wherein it gets activated locally for reasons stated above, unambiguously translates into an advantageous therapeutic property, namely to activate PG predominantly in the vicinity of the target without systemic PG activation in the overall circulatory system, unlike a PG activator that activates PG as soon as it is introduced into the circulatory system.

Further, by virtue of the elements of design employed in the engineering of an initial PG activation time lag -- a vital component of our construct – the claimed recombinant plasminogen activator is clearly distinguishable from that of Malke et al. which discloses a fusion of plasminogen kringle domains at the N-terminus of SK with no reported alteration in PG activation characteristics compared to native SK. In addition, thespecification discloses designs that demonstrate different lag periods for PG activation, which underlies the fact that the

modulation of the time lag is also a distinct feature of our invention, a property that is obviously of tremendous significance in designing and then choosing a tailor-made thrombolytic protein for different clinical scenarios.

The Examiner must appreciate that the cited arts are of years 1990 and 1992. The inventors have put several years of research to come out with the invention of the instant application. Had it been obvious for a person skilled in the art, then, would not the much-awaited instant work come out long back? The area of invention is a very 'hot' area of research. This is so because millions of people die from heart attacks and other related disease conditions, and the current thrombolytic drugs, including streptokinase, have severe side-effects emanating from their lack of clot-specific (i.e. systemic) plasminogen activation mode of action. Hence, an improved thrombolytic with a time-delayed, target-activable mode of action as described in the instant invention can be of great value. The instant work is a major breakthrough in the field. A person skilled in the art would immediately realize the significance of the instant work.

The examiner is not free to ignore the assertion and documentation of a surprising result. *In re Soni*, 54 F.3d 746, 34 USPQ2d 1684 (Fed. Cir. 1995). Nowhere does the Examiner provide an analysis of this issue.

Respectfully submitted,

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